



Research Article

ANTIFUNGAL ACTIVITY OF AQUEOUS AND HYDROALCOHOLIC EXTRACTS OF DESERT PLANT DHAMASA AND THEIR COMPARISON BY STATISTICAL ANALYSIS

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ABSTRACT

Herbal plant Dhamasa is widely distributed in deserts and dry areas of India, Pakistan to tropical Africa. Botanical name of Dhamasa is *Fagonia schweinfurthii* Hadidi (Family-Zygophyllaceae). Traditionally it is boiled in water and its bath is taken for allergies and other skin diseases. In this study the antifungal activity of the aqueous and hydroalcoholic extracts of Dhamasa were evaluated. Aqueous extract was prepared by decoction method and hydroalcoholic extract was prepared by soxhletion method. The evaluation of antifungal activity of plant extracts was determined as the minimum inhibitory concentration (MIC) followed by cup plate method against three fungal strains. Inherent antifungal activity of aqueous as well as hydro-alcoholic extract of Dhamasa was found. For the comparison of results Student's t test was applied on the values of diameters of zones of inhibitions produced by aqueous and hydroalcoholic extracts. The result indicated that aqueous as fungal strains. Statistical analysis of results reveal that there was no significant difference in the antifungal potential of aqueous as well as hydroalcoholic extract possess almost equal antifungal potential against selected. These extract can be used in various antifungal topical preparations.

Keywords: Antimicrobial study, Fungal pathogens, Student's t test

INTRODUCTION

All parts of plant synthesize various chemicals which metabolize their physiological activities. These phytochemicals are often used to cure the diseases in herbal and homeopathic system of medicines. Now a day most of the people like to use the traditional methods of medicines to cure general diseases¹.

In the past few decades, a worldwide increase in the incidences of fungal infections has been observed. The majority of clinically used anti-fungals have various drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to the emergence of resistant strains. The challenge has been felt to develop effective strategies for the treatment of candidiasis and other fungal diseases, considering the increase in opportunistic fungal infections in immuno-compromised patients either due to cancer chemotherapy or due to the indiscriminate use of antibiotics².

Due to the development of resistance in known fungal pathogens and the emergence of fungal pathogens intrinsically resistant to the currently available antifungals, it is important that novel antifungal agents be identified and developed. Natural plant extracts may provide an alternative to chemical preservatives. Over the years much effort has been devoted to the search for new antifungal materials from natural sources. Number of plants were investigated for their antifungal and antibacterial activity like *Allium sativum* L., *Allium cepa* L., *Allium porrum* L. against *Aspergillus niger*³, origanum oil against *Candida albicans*⁴, *Anogeissus leiocarpus* and *Terminalia avicennioides* against *Aspergillus niger*, *Aspergillus fumigatus*, *Microsporium audouinii* and *Trichophyton rubrum*⁵, *Alpinia officinarum* against the *Bacillus cereus*, *Staphylococcus aureus*,

Pseudomonas auroginosa, *Escherichia coli*⁶, *Origanum vulgare* L⁷, *Syzygium cumini* against *Ascochyta rabiei*⁸ and *Polygonum acuminatum* showed to possess antifungal properties against yeasts as well as dermatophytes but not against *Aspergillus* spp⁹.

Traditionally the powder made up of whole plant of Dhamasa is dusted on boils and skin eruption. Whole plant is boiled in water and its bath is taken for allergies and other skin diseases¹⁰. The significant antihistaminic activity was found in extract and formulation of *Fagonia schweinfurthii* Hadidi¹¹. For the analytical estimation a simple rapid, accurate, precise and economic spectrophotometric method was developed for aqueous extract of *Fagonia schweinfurthii* Hadidi¹². The aim of present study was to evaluate the antifungal potential of herbal plant Dhamasa (Family: Zygophyllaceae).

MATERIALS AND METHODS

Plant Material

The plant was collected from Jodhpur region of Rajasthan, India and was authenticated from Botanical Survey of India, Jodhpur, Rajasthan (India). Voucher specimens and herbarium sheet was kept in the institute for further references.

Preparation of Extracts¹³

Aqueous Extract

Fresh plant of Dhamasa was shade dried and grounded to prepare a moderately coarse powder. The extraction was carried out by decoction method with water at 40°C. The extract was

filtered through a 45 µm membrane filter, and the filtrate was dried with help of a vacuum evaporator. The crude extract was stored in a desiccator.

Hydroalcoholic (HA) Extract

A Soxhlet apparatus was used for the extraction. The collected plant was shade dried and powdered. 20 gm of dried powder was packed in thimble and then extracted with a mixture of water and ethanol (50:50). The extract was filtered through 45 µm membrane filter, and the filtrate was dried in a vacuum evaporator. The crude extract was stored in desiccator.

Test Microorganisms and Culture Media

Strains of fungi were obtained from MTCC (Microbial Type Culture Collection) Chandigarh, India. *Candida albicans* (MTCC 183), *Aspergillus niger* (MTCC 281) and *Aspergillus*

fumigatus (MTCC 870) were selected for screening of Antifungal activity of aqueous and hydroalcoholic extracts.

C. albicans, *C. fumigatus* and *A. niger* were grown in Sabouraud Dextrose Broth and Sabouraud Dextrose Agar. The concentrations of all fungal suspensions were adjusted to 10⁷ cells/mL, before experiment.

Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration was determined according to procedure given in Cooper and Gunn's Tutorial Pharmacy by S.J. Carter Carter, 2006¹⁴. For the study 500 µg/ml stock solution of each extract was prepared and suitably diluted in clean test tubes with inoculum and nutrient broth. Table No. 1 illustrated the arrangement of dilutions in seven test tubes for determination of MIC

Table 1: Determination of Minimal Inhibitory Concentration

Tubes content (in ml)	Tube Number						
	1	2	3	4	5	6	7
Broth	5	5	5	5	5	5	5
Extract	4	3.5	3	2.5	2	0	4
Sterile water	0.8	1.3	1.8	2.3	2.8	4.8	1
Inoculum	0.2	0.2	0.2	0.2	0.2	0.2	0.0
Total volume	10	10	10	10	10	10	10

Contents of all the test tubes were mixed using vortex shaker and the tubes were incubated at 28° C for 24 h. The turbidity in each test tube was observed after 24 h.

Antifungal Activity Test^{3,15}

Cup plate method was used for evaluation of antifungal activity of extracts. Sterile Sabouraud Dextrose Agar at 43-45°C was poured into the sterilized petri plates (7 cm diameter). Then the agar was allowed to solidify for 1 h. 0.1 mL of different fungal culture inoculums were applied on each plate. Inoculum was evenly spread on agar using a glass rod spreader. For agar well diffusion method, a well was prepared in each of the plates with the help of a cup-borer (0.8 cm diameter). 100 µl of each of the test compound having 2000 µg/ml concentration was filled into individual well. The concentration of test compound was selected according to MIC. The plates were incubated for 24 h at

28° C. At the end of incubation period, the zones of inhibitions formed in the medium were measured. All experiments were performed in six replicates.

Statistical Analysis

Statistical analysis was done by applying Student's t test on the values of diameters of zones of inhibitions.

RESULTS

Table 2 and 3 represent the values of MIC (in µg/ml) and diameters of zones of inhibitions (in cm) respectively observed for different fungal strains. Figure 1 shows the comparative bar graph of diameter of zone of inhibition of extracts against different fungal species.

Table 2: Minimum inhibitory concentration of extracts against different fungal species

S. No.	Fungal Strains	MIC of Aq. Extract (µg/ml)	MIC of HA extract (µg/ml)
1.	<i>Candida albicans</i>	100	100
2.	<i>Aspergillus niger</i>	125	100
3.	<i>Aspergillus fumigatus</i>	125	100

Table 3: Diameters of zones of inhibitions of extracts against different fungal species

S. No.	Fungal Strains	Diameter of zone of Inhibition (in mm) (For Aq. extract)	Diameter of zone of Inhibition (in mm) (For HA extract)
1.	<i>Candida albicans</i>	38 ± 0.7	39 ± 1.7
2.	<i>Aspergillus niger</i>	33 ± 1.7	33 ± 0.7
3.	<i>Aspergillus fumigatus</i>	32 ± 1.1	33 ± 1.0

n=6

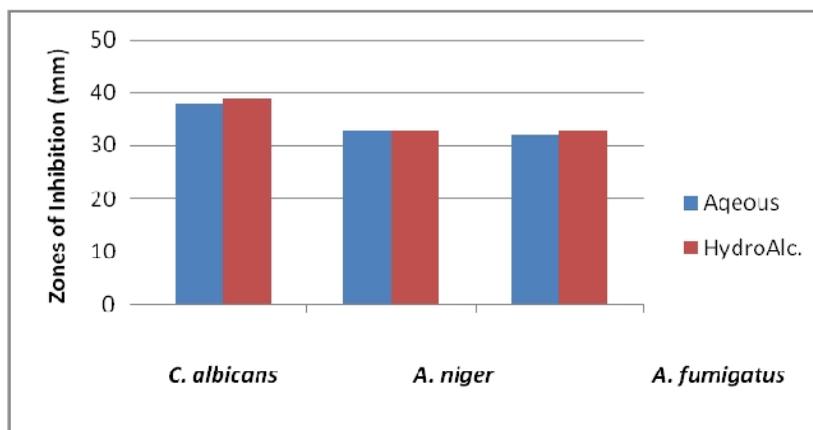


Figure 1: Diameter of zone of inhibition of extracts against different fungal species

DISCUSSION

This study evaluated the inherent antifungal activity of aqueous as well as hydro-alcoholic extract of *Fagonia schweinfurthii hadidi* was found. Moreover, these current findings were similar with those of some previous reports, where antifungal activity of crude extract from *Cassia alata* (Leguminosae) were demonstrated against *Trycophyton rubrum*, *Trycophyтом mentagrophytes* and *Microsporium gypseum*^{16,17}. Aqueous extract and methanolic extract of herb *Lantana camara* was shown antifungal activity against *Aspergillus fumigatus* and *A. flavus*¹⁸. Allium plants have antifungal effects to *A. niger*. The most inhibitory plant was garlic, followed by onion and leek. Ethyl alcohol extracts of the garlic and onion significantly show inhibitor effect against *A. niger*. Also, inhibitor activities were observed for aqueous extracts of garlic and leek. Acetone extracts of onion and leek did not show any effect on *A. niger*³.

Student's t test was apply on observations of diameter of zone of inhibition by aqueous extract and hydroalcoholic extract for three fungal strains. In study it was found that t calculated value is less than t tabulated value so that null hypothesis was accepted, there for it can be say that no significance difference was found between the antimicrobial study of aqueous extract and hydroalcoholic extract at 5% level of significance and degree of freedom was 4. From the obtained results it can be concluded that aqueous extract has equal antifungal activity with hydroalcoholic extract. So aqueous extract can be incorporated into topical medications like cream, lotion, ointment for antifungal therapy.

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